

# Rifampicin resistance in *Mycobacterium tuberculosis* — rapid detection and implications in chemotherapy

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**Objectives.** Tuberculosis treatment and susceptibility testing are cumbersome, especially in the case of multidrug-resistant (MDR) *Mycobacterium tuberculosis*. It is known that mutations in the *rpoB* gene of *M. tuberculosis* lead to resistance to rifampicin (RMP). In this study, an attempt was made to apply molecular techniques for rapid detection of antibiotic resistance in clinical isolates of *M. tuberculosis*.

**Design, settings and subjects.** RMP-resistant clinical isolates of *M. tuberculosis* from South Africa ( $N = 120$ ) with unique resistant patterns were selected for calculation of resistance frequencies, and 74 MDR isolates of *M. tuberculosis* from different geographical origins were used for microbiological and molecular analysis. The polymerase chain reaction (PCR) technique was applied for amplification of a previously described region around a cluster of mutations in the *rpoB* gene, and single-stranded conformational polymorphism (SSCP) analysis was optimised to screen for mutations in the amplified region.

**Results.** The results showed that an optimised PCR-SSCP procedure could detect a cluster of mutations in the *rpoB* gene (for RMP resistance) in 95% of RMP-resistant isolates. This procedure could therefore be used in the prediction of RMP resistance.

Evidence was obtained that these mutations can be screened for directly from BACTEC cultures or even directly from Ziehl-Neelsen-positive sputum samples. Statistical analysis also showed that this locus can be used to predict the presence of an MDR isolate, which may have important implications in decisions concerning chemotherapy.

**Conclusions.** It is currently not feasible to test all tuberculosis cases, but application of the PCR-SSCP technology in the prediction of multidrug resistance in *M. tuberculosis* isolates may be important in patients, especially where frequencies are high for drug-resistant isolates. This methodology could reduce the time required for sensitivity testing from approximately 6 - 12 weeks to a few days.

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Tuberculosis treatment regimens including rifampicin (RMP) and isoniazid (INH) have been complicated by an increasing frequency of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis*. The prevalence of tuberculosis before the 1980s, which is considered to be a turning point in the struggle with the tubercle bacillus, indicated a decline in the disease.<sup>1</sup> However, owing to limited understanding of the disease and poor compliance, a resurgence since the middle to late 1980s occurred.<sup>2</sup> Of concern is the emergence of drug-resistant strains during the last few years, and efforts have been made to unravel the mechanisms of action and to understand the molecular basis of resistance to these antibiotics. Deviations in structural genes encoding target-proteins may lead to differences in secondary structure of such proteins, and therefore loss of functionality. These differences may cause insufficient binding of the antibiotic to its target, and ultimately lead to antibiotic resistance. It is generally accepted that the phenotypic outgrowth of the antibiotic-resistant organisms is the result of selective pressures caused by the antibiotic.

It is now possible to detect many causative mutations with the aid of molecular technology. Much of the detection has been done using a polymerase chain reaction (PCR)-based technique, single-stranded conformation polymorphism (SSCP), to enable the detection of single-base substitutions, as well as small deletions and/or insertions.<sup>3</sup> This technique has been widely applied in the investigation of drug resistance in tuberculosis as well as in human genetic diseases.<sup>3-5</sup>

It is known from studies on *Escherichia coli* that RMP is directed towards the  $\beta$ -subunit of RNA polymerase, encoded by the *rpoB* gene.<sup>6</sup> Comparison of the primary structure of the *rpoB* proteins from several bacteria led to the identification of six highly conserved regions, including a cluster near the centre of the *rpoB* gene. This region is frequently mutated, and by using PCR-SSCP analysis, together with nucleotide sequencing analysis, mutations involving eight conserved amino acids have been identified in this region in ~97% of RMP-resistant isolates of *E. coli*, *M. smegmatis* and *M. tuberculosis*.<sup>6-8</sup> These mutations were all clustered within a 23-amino acid-encoding region of the *rpoB* gene, and encode resistance to RMP in *M. tuberculosis*.<sup>8</sup> The same mutations were not found in isolates susceptible to RMP.

Susceptibility testing of antibiotics in slow-growing organisms is cumbersome and requires pure isolates of the test organism. The aim of this study was to optimise the SSCP procedure for detection of *rpoB* gene mutations, and to use this gene locus as molecular marker together with MDR profiles of clinical isolates of *M. tuberculosis* to assess

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whether this protocol could be used to predict the presence of a MDR strain of *M. tuberculosis* in biological samples.

## Material and methods

### Clinical isolates

The records of 120 RMP-resistant clinical isolates with unique resistance patterns, determined by culture testing,<sup>9</sup> were selected from the database of the National Tuberculosis Research Programme and used in a statistical calculation. Cultures and DNA of 74 RMP-resistant strains of *M. tuberculosis* with different resistance patterns were used in this study, for further microbiological and molecular analysis (Table I).

Minimal inhibitory concentrations (MICs, in µg/ml) for rifampicin were determined by the agar dilution method,<sup>9</sup> by incorporating serial twofold dilutions of rifampicin into Middlebrook 7H10 agar medium. The inoculates were prepared from 10-day-old cultures, diluted to a concentration of approximately 10<sup>6</sup> colony-forming units per millilitre. Ten microlitres of each culture were then inoculated (in duplicate) onto the drug-containing and drug-free control plates, sealed in plastic bags and incubated at 37°C for 21 days. The lowest concentration of the antibiotic inhibiting growth of more than 99% of the bacterial population was considered the MIC.

A scrape of each culture was suspended individually in ~300 µl of sterile distilled water or saline. Alternatively, DNA was extracted from some cultures using a standard protocol.<sup>10</sup> These two different templates were used for PCR amplification.

### PCR amplification

#### Oligonucleotide primers and PCR procedure

Primer set TR8/TR9 (TR8 5' TGCACGTCGCGGACCTCCA, TR9 5' TCGCCGCGATCAAGGAGT) was synthesised by Genosys, UK, according to the *rpoB* gene sequence (GenBank accession number L05910), for PCR amplification of a 157bp fragment of the *rpoB* gene.

DNA amplification reaction mixtures (per reaction of 95 µl) consisted of 1 x *Taq* buffer, 2.5 mM MgCl<sub>2</sub>, 200 µM each dNTP (dATP, dCTP, dGTP and dTTP, respectively), 10 µM each of the 5' and 3' oligonucleotide primer and 1.5 U *Taq* polymerase. Five microlitres of template DNA (~1 µg) were inoculated in the reaction mix. Cycling temperatures were as follows: 93°C for 3 minutes, followed by 35 cycles at 93°C for 1 minute, 58°C for 2 minutes and 72°C for 2 minutes, and a final extension cycle at 72°C for 10 minutes. Cycling was done in a Multigene PCR cycler (MedTech, Cape Town). Confirmation of PCR amplification was done using 10 µl amplified product, electrophoresed on 12% PAGE mini-gels at 100 V (constant) for 1½ hours in 1 x TBE (10 x TBE = 900 mM each Tris and boric acid, and 25 mM EDTA) buffer, using a BioRad Mini Protean II electrophoresis system and ethidium bromide staining/UV illumination for detection. This work was carried out in a PCR-equipped laboratory, and great care was taken to avoid false results due to contamination of amplified fragments.<sup>11</sup>

**Table I. Characteristics and molecular data of RMP-resistant clinical isolates**

Isolate	Resistance pattern	MIC, RMP (in µg/ml, at 21 days)	Molecular data mut
<b>South Africa</b>			
92/3	INH, RMP, ETH	NA	+
92/4	INH, RMP, SM	> 2.0	+
92/5	INH, RMP, SM, ETH, EMB	> 2.0	+
92/6	INH, RMP, SM	> 2.0	+
92/7	INH, RMP, SM	NA	+
93/1	INH, RMP, SM, EMB, PZA	> 2.0	+
93/8	INH, RMP, SM, EMB, PZA	1.25	+
93/16	INH, RMP, SM, ETH, EMB, PZA	> 2.0	+
93/17	INH, RMP, SM, PZA	> 2.0	+
93/19	INH, RMP, SM	> 2.0	+
93/20	INH, RMP, SM, EMB	2.5	+
93/21	INH, RMP, SM, ETH, EM	1.25	+
TB1523/92	INH, RMP, SM	> 2.0	+
1672	INH, RMP, SM	NA	+
1873	INH, RMP, SM, ETH	> 2.0	-*
1876	INH, RMP, SM	> 2.0	+
1893	INH, RMP, SM, ETH, EMB	20	+
1984	INH, RMP, EMB	20	+
2066	INH, RMP, SM, ETH, EMB	10	+
2211	INH, RMP	> 2.0	+
2364	INH, RMP, SM	NA	+
TB0019/93	INH, RMP, SM, ETH	> 2.0	+
0150	INH, RMP, SM, ETH	> 2.0	+
0202	INH, RMP, SM	> 2.0	+
0225	INH, RMP, EMB	> 2.0	+
0249	INH, RMP, EMB	> 2.0	+
0271	INH, RMP, SM	> 2.0	+
0287	INH, RMP	> 2.0	+
0368	INH, RMP, ETH	> 2.0	+
0416	INH, RMP	> 2.0	+
0531	INH, RMP, SM	> 2.0	+
0546	INH, RMP, SM, EMB	> 2.0	+
0547	INH, RMP, SM	> 2.0	+
0552	INH, RMP, SM	5.0	+
0565	INH, RMP, SM, ETH, EMB	NA	+
0587	INH, RMP, SM	> 2.0	+
0651	INH, RMP, SM	> 2.0	-
0891	INH, RMP	> 2.0	-
52	INH, RMP	> 100	+
335	INH, RMP, ETH	> 100	+
219	INH, RMP	> 2.0	+
310	INH, RMP	> 100	-
331	INH, RMP	> 100	-
257	INH, RMP	> 100	-
508	INH, RMP	> 100	+
V10	INH, RMP	> 100	+
V14	RMP	> 100	+
V26	INH, RMP, SM	> 100	-
V108	INH, RMP, SM, Kanam, Th	> 2.0	+
V113	INH, RMP	> 64	+
V117	INH, RMP, SM	> 100	+
V122	INH, RMP, SM, Kanam, Th	> 100	+
V134	INH, RMP, Cyclo	> 100	+
V142	INH, RMP, SM, Th	> 100	+
V146	INH, RMP, SM, EMB, Cyclo	> 100	+
M1	INH, RMP, SM	> 100	+
M2	INH, RMP	> 100	+
M3	INH, RMP, SM, EMB, Kanam, Th	> 100	-
M4	INH, RMP, Kanam	> 100	-
M5	INH, RMP	> 100	+
M6	INH, RMP, SM, EMB, Kanam	> 100	-
M7	INH, RMP, SM, Kanam	> 100	+
M8	INH, RMP, SM, Kanam, Th	> 100	-
M9	INH, RMP	> 100	+
M10	INH, RMP, SM	> 100	-
<b>Health Department, Arkansas</b>			
1046	INH, RMP	1.0	+
<b>Health Department, Texas</b>			
11306	INH, RMP, EMB	> 2.0	+
113989	INH, RMP, EMB	NA	-
113035	INH, RMP, EMB	> 2.0	+
113716	INH, RMP, EMB	> 2.0	+
113560	INH, EMB	NA	-
<b>Switzerland</b>			
TB173	INH, RMP	5.0	-
TB160	INH, RMP	> 2.0	+
TB149	INH, RMP	6.0	-
<b>Control</b>			
H <sub>2</sub> Rv	fully sensitive	< 1.0	-

\* No gene deviations.

INH = isoniazid; RMP = rifampicin; ETH = ethionamide; EMB = ethambutol; PZA = pyrazinamide; Kanam = kanamycin; Th = thiacetazone; SM = streptomycin; Cyclo = cycloserine; MIC = minimal inhibitory concentration; mut = mutation; NA = no data available.



## Mutation analysis

A standard protocol, previously described for mutational analysis by SSCP, was used initially.<sup>3</sup> Equal volumes of PCR product (10 µl each) and stop solution (containing 95% formamide, 20 mM EDTA, and 0.005% each of bromophenol blue and xylene cyanol FF) were mixed and heat-denatured at 95°C for 10 minutes, after which the mix was snap-cooled on ice prior to loading onto the gel.

Optimisation of the SSCP technique for detection of *rpoB* mutations was done with 20 isolates (isolates TB1523/92 to TB0546/93, Table I). Different combinations of parameters were used in the mutational analysis of the amplified denatured PCR products. The SSCP gels (1 mm in thickness) consisted of 6% polyacrylamide (a stock of 30% acrylamide was used, containing 29% acrylamide and 1% N'N' methylene bis-acrylamide), 0.6 x TBE buffer and 5% or 0% glycerol, respectively, polymerised with 4.2 ml 3% ammonium persulphate and 10% TEMED in a total volume of 200 ml. FMC Gelbond iso-electric focusing film was used to assist in the handling of the gels. The gels were electrophoresed in 0.6 x TBE buffer at 50 W or 25 W (constant power) at room temperature, with cooling from a

bench-top fan, or at 0°C for 4 - 5 hours respectively. After electrophoresis, the gels were disassembled, and fixed for 10 minutes in a buffer containing 10% ethanol and 0.05% acetic acid. The gels were subsequently placed in a 0.1% silver nitrate buffer for 10 minutes.

After washing twice in distilled water, staining was done on a horizontal orbital shaker in a buffer containing 0.1M sodium hydroxide, 1.5M sodium borohydride and 0.004% formaldehyde for 20 minutes (or until the bands were visible). Final fixing of the bands was done in a 0.75% sodium carbonate buffer for 10 minutes.

## Preparation of BACTEC cultures and sputum samples for mutational screening

A scrape of isolate TB0287/93, resistant to RMP and INH, and containing a mutation in the *rpoB* gene (Table I), was suspended in 200 µl of phosphate-buffered saline (PBS). Different ratios of the isolate and the control strain, H<sub>37</sub>Rv, were inoculated into BACTEC medium (Table II). Growth indices (GIs) were read at 24-hourly intervals on days 0, 1, 2

Table II. BACTEC procedure for detection of mutations — PCR amplification and SSCP analysis after 24-hourly growth periods of RMP-resistant isolate TB0287/93, with H<sub>37</sub>Rv as control, in BACTEC medium

Sample	Isolate	Sample contents of BACTEC bottle	Day	Growth index	PCR result	SSCP result
1	H <sub>37</sub> Rv	50 µl both isolate and saline	0	36	-	ND
			1	123	Pos.	-
			2	280	ND	ND
			3	622	ND	ND
			4	≥ 999	ND	ND
2	TB0287/93	50 µl both isolate and saline	0	9	-	ND
			1	20	Pos.	Pos.
			2	35	ND	ND
			3	68	ND	ND
			4	135	ND	ND
3a	H <sub>37</sub> Rv TB0287/93	50 µl both isolates (1:1)	0	69	-	ND
			1	241	Pos.	Pos.
			2	453	ND	ND
			3	799	ND	ND
			4	≥ 999	ND	ND
b	"	(2:1)	0	81	-	ND
			1	288	Pos.	Pos.
			2	666	ND	ND
			3	≥ 999	ND	ND
			4	ND	ND	ND
c	"	(4:1)	0	56	-	ND
			1	211	Pos.	Pos.
			2	461	ND	ND
			3	916	ND	ND
			4	≥ 999	ND	ND
d	"	(8:1)	0	14	-	ND
			1	39	Pos.	Pos.
			2	89	ND	ND
			3	237	ND	ND
			4	596	ND	ND
e	"	(16:1)	0	19	-	ND
			1	50	Pos.	Pos.
			2	98	ND	ND
			3	264	ND	ND
			4	587	ND	ND

- = no amplification; Pos. = positive result; ND = not done.



and 3 respectively, using a BACTEC apparatus (Becton-Dickenson, BACTEC 460, Johnston Laboratories, Md, USA) after incubation at 37°C, or until GIs exceeded a reading of 999. After measurement of GIs, 200 µl volumes were removed from each BACTEC culture, and used for PCR-SSCP analysis. Five microlitres of each were inoculated in respective PCR amplification tubes prior to analysis.

Inhibitors of PCR-reactions in four Ziehl-Neelsen (ZN)-positive sputum samples were removed by a sucrose procedure,<sup>12</sup> and the resulting pellets were used directly for PCR amplification of a 204bp fragment of the *katG* gene. Amplification of this gene segment was used as a model, and the PCR procedure was the same as described previously.<sup>5</sup> Purified DNA from *M. tuberculosis* strain H<sub>37</sub>Rv was used as control.

## Results

### Detection of mutations in the *rpoB* gene

Pure cultures, or alternatively DNA, of *M. tuberculosis* isolates resistant to RMP (Table I) were used for PCR amplification of *rpoB*, and subsequently SSCP analysis was done for mutational screening. Different combinations of conditions were investigated to optimise the SSCP procedure for detection of mutations. Using a 'standard set' of conditions, including 5% glycerol in the 6% SSCP gels and electrophoresis performed at 50 W for 4 - 5 hours at room temperature, we were able to detect mutations in the *rpoB* genes of 70% of the isolates. Fig. 1 is a sample result. Since a higher incidence of mutations was expected for this gene locus, different electrophoretic conditions were subsequently evaluated for isolates TB1523/92 to TB0546/93 (Table I). These conditions included gels without glycerol, different percentages of glycerol and electrophoresis at different temperatures and voltage gradients. Electrophoresis at 4°C at 25 W for 4 - 5 hours, without glycerol, gave the best result. Additional mobility shifts were detected in this group of samples with this procedure. Therefore, by using a combination of these two procedures, mutations in *rpoB* could be detected in ~95% of the RMP-resistant isolates tested by SSCP analysis.

### Statistical analysis

Most MDR *M. tuberculosis* isolates are resistant to rifampicin. All the isolates (N = 120) used in this study were either resistant to one (RMP) or more antibiotics. Statistical analysis of the proportions was done under a binomial distribution curve, using a scientific table for statistical values in a population.<sup>13</sup> This analysis showed that all (100%) of the RMP-resistant isolates were resistant to INH and 10% were also resistant to streptomycin (SM). Much lower percentages for the other antibiotics (6%, 6%, 4% and 1.5%) were obtained for cycloserine (Cyclo), kanamycin (Kanam), ethambutol (EMB) and thiacetazone (Thiacet) respectively. P-values were calculated and occurred between 96.97 and 100 for 95% and 99% confidence limits respectively for INH resistance. For the other antibiotics, the P-values were not significant.

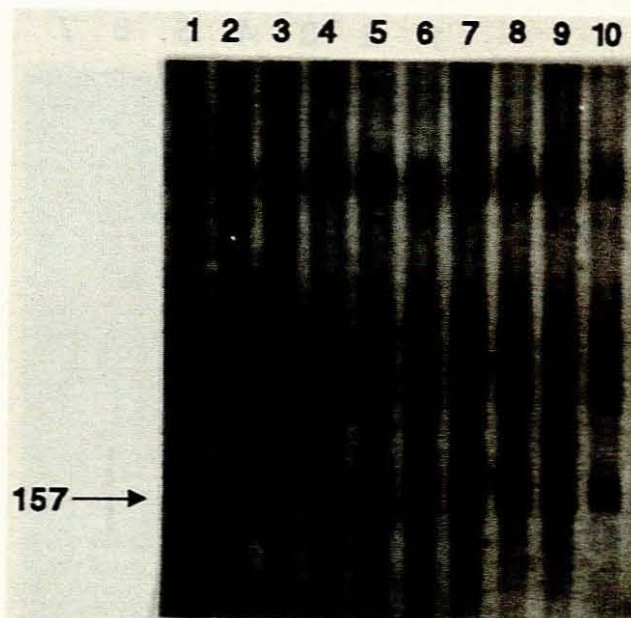


Fig. 1. Mutations in the *rpoB* gene of *M. tuberculosis*. SSCP analysis was done using a 'standard set' of conditions as described under 'Materials and methods'. Lanes 1 to 9 represent PCR amplification product of cultures of RMP-resistant clinical isolates, and the control strain H<sub>37</sub>Rv in lane 10. The molecular weight marker, øX174/HaeIII was used, although not indicated in this figure.

### Detection of mutations directly from BACTEC cultures

Previous reports, including the mutational analyses described above, have so far made use of pure cultures or sub-cultures for amplification of the *M. tuberculosis* genes. Implementation of a combination of a standard culturing method together with a molecular technique was attempted for shortened screening for drug resistance of *M. tuberculosis*-containing clinical samples.

The results indicated that amplification of the *rpoB* gene fragment could be obtained from BACTEC cultures with GIs ≥ 100, which were obtained on day 1 (Fig. 2). Once positive amplification could be obtained, SSCP analysis was possible. Results showed that even at a dilution of 1:16, a mobility shift was visible (lane 7, Fig. 2). This result indicated that it is possible to detect *rpoB* gene mutations by the SSCP procedure directly from cultures in BACTEC medium after a relatively short culture period.

### Direct detection of mutations from sputum samples

Four ZN-positive sputum samples were used. Templates for the amplification of a fragment of the *katG* gene, including codon 463,<sup>5</sup> were prepared by a sucrose method described previously.<sup>12</sup> Positive amplification was possible using these isolates, after which SSCP analysis was done. The result (Fig. 3) showed that PCR-SSCP analysis can be done directly from raw sputum samples, free from PCR amplification inhibitors and without prior DNA purification. No band mobility shifts were detected for these particular samples with SSCP analysis, compared to the control H<sub>37</sub>Rv.



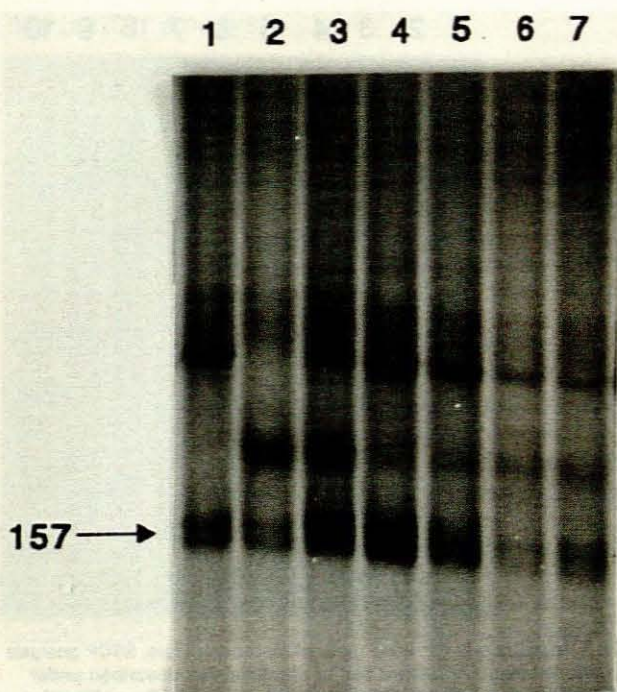


Fig. 2. Early detection of mutations in the *rpoB* gene of *M. tuberculosis*, obtained from PCR amplification products after short growth (1 day) in BACTEC culture medium. A RMP resistant strain, TB0287/93, previously shown to contain a mutation in this gene, was used. Lane 1: control strain H<sub>37</sub>Rv, lane 2: TB0287/93, lanes 3 - 7: dilutions in different ratios of the control and resistant isolates (1:1, 2:1, 4:1, 8:1, and 16:1, respectively).  $\phi$ X174/*Hae*III was used as molecular weight marker (not shown).

## Discussion

The use of conventional culturing methods in the diagnosis and susceptibility testing of clinical isolates of *M. tuberculosis* is protracted, and can take up to 12 weeks before a prediction of a resistance pattern can be made. This has negative consequences for the treatment of infected individuals, and can contribute towards spread of MDR organisms. Ideally, instead of culture detection for susceptibility testing of tubercle bacilli, it would be more useful to have a procedure which could shorten these procedures. It has previously been shown that > 90% of clinical isolates resistant to RMP have mutations in the *rpoB* gene.<sup>8</sup> This allows the attractive opportunity of using this locus as a marker for RMP resistance. However, results from the previous studies were collectively obtained from both SSCP analysis and sequence data. Results from this study showed that by using a combination of two optimised SSCP procedures, it was possible to detect mutations in 95% of 20 RMP-resistant clinical isolates of *M. tuberculosis*. It is possible that the remaining 5% of the samples analysed, not showing SSCP mobility shifts with any of these two optimised procedures, might not have mutations in this amplified region of *rpoB*. Alternatively, these two procedures might not be optimised for detection of some uncommon mutations. Other methods, such as dideoxy-fingerprinting (ddF)<sup>14</sup> and denaturing gradient gel electrophoresis (DDGE),<sup>15</sup> might assist in detection of these mutations, if present.

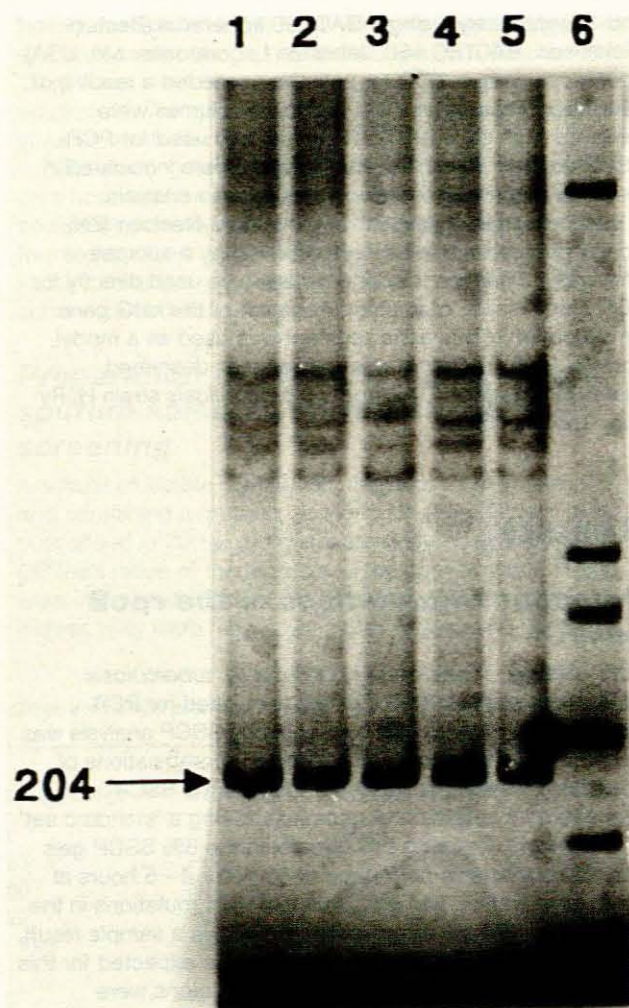


Fig. 3. PCR amplification products of a fragment of the *katG* gene from four ZN-positive sputum samples were analysed by SSCP. Lanes 1 - 4 represent the sputum samples, and lane 5 the control H<sub>37</sub>Rv. The molecular marker  $\phi$ X174/*Hae*III can be seen in lane 6.

For diagnostic purposes it is important to evaluate these procedures, since automated sequencing is not economical or practical for all institutions.

The results suggest that it is not only possible to use mutations in the *rpoB* gene to predict RMP resistance, but since there is a 95% chance that the particular organisms are also INH-resistant, this locus (*rpoB*) may also be used in the prediction of MDR strains. It was also shown in this study that culture may be unnecessary, and that mutational screening can be done after short culture periods in BACTEC medium, or even directly from sputum samples. This may have immediate implications for the decision on chemotherapy. It is currently not feasible to test all tuberculosis cases, and it is recommended that only high-risk cases be selected.

A recent survey on drug resistance in the Western Cape<sup>16</sup> indicated figures of 10.8% and 4.2% for acquired resistance, and an overall incidence of 6.8% and 2.4% ( $N = 7\ 266$ ) for INH and RMP respectively, when both initial and acquired resistance were taken into account. From this it can be predicted that approximately 174 patients were infected with MDR strains of *M. tuberculosis*, and the



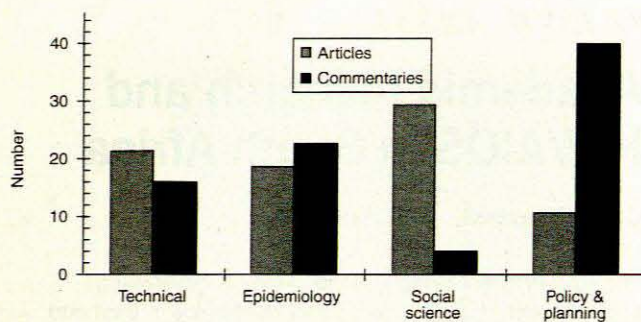


Fig. 1. The number of articles (including reviews) and commentaries (including editorials, letters, reports and briefings) devoted to four main categories of work: (i) technical — including associated diseases, assays, clinical features and diagnosis; (ii) epidemiology — including estimates of incidence and prevalence, predictions and modelling; (iii) social science — including behaviour, counselling, education, knowledge; (iv) policy and planning. Included in this breakdown are 82 refereed articles and 84 commentaries.

Fig. 2 shows changes in the emphasis of research over time. The number of papers devoted to technical matters has remained steady, the number of epidemiological studies increased after the mid-1980s but the contribution of social science has increased dramatically over the 12-year period, reflecting the increasing awareness that technical solutions are unlikely to be found in the near future and that greater emphasis needs to be placed on social issues that were neglected in the early years of the epidemic.

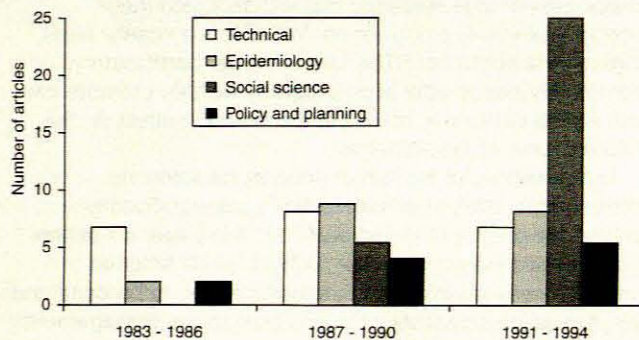


Fig. 2. The number of articles and reviews (excluding commentaries) published on HIV/AIDS in South Africa according to four categories for 4-year periods.

## Technical studies

Since the diagnosis of the first AIDS cases in South Africa<sup>2</sup> a number of technical studies have been carried out. These have been concerned with the safety of blood supplies, diseases associated with HIV infection, and clinical features of AIDS. There has been considerable debate about the suitability of various tests for HIV infection.<sup>3</sup>

The experience in South Africa is similar to that in most African countries:<sup>4</sup> tuberculosis is the most common complicating infection, followed by acute pneumonia; *Pneumocystis carinii* pneumonia is rare and *Herpes zoster* is often the first sign of HIV infection. Early studies showed that South Africans with Kaposi's sarcoma do not have significant underlying immunodeficiency or associated

opportunistic infections.<sup>5</sup> Other studies have included retinal complications,<sup>6</sup> neurological manifestations of HIV-2<sup>7</sup> and the importance of cancers, especially Kaposi's sarcoma, in HIV-positive people.<sup>8</sup> Serological markers of STDs have been used to show that pregnant women with HIV-1 infection are more likely to show evidence of STD infection than uninfected women<sup>9</sup> and that there is a high risk of STDs among asymptomatic HIV-positive female blood donors.<sup>10</sup>

## Epidemiology

The fragmented nature of South African society has led to a series of epidemics, first among the gay community, then people suffering from haemophilia and requiring blood products, then the urban working classes, and now those living in rural areas.

During the latter half of the 1980s, several estimates were made of the prevalence of HIV infection among gay men; this was then thought to be between 10% and 15%.<sup>11</sup> Because these mainly middle class, educated people were affected early on and have experienced the full force of the epidemic, they may be the one group among whom the prevalence of HIV is no longer increasing, and the number of AIDS cases reported among homosexual and bisexual men and drug users levelled off at about 40 per 6 months in 1990.<sup>12</sup>

In the early 1980s, a mini-epidemic was experienced among haemophiliacs of whom 39 became infected with HIV, apparently as a result of receiving American factor VIII concentrate.<sup>13</sup> Subsequent blood transfusions are thought to be safe, however, and this is not expected to present significant risks in the future.<sup>14</sup> Extensive testing has subsequently been done on blood donors and during the first 6 months of 1988, only 0.005% (46 people) were found to be HIV-positive.<sup>11</sup> While blood donors under-represent high-risk groups, it is clear that the general level of HIV infection was still low in the late 1980s.

Although the data are less precise for intravenous drug users, they too showed little evidence of HIV infection in the 1980s.<sup>15</sup> The first confirmed case of AIDS clearly attributable to intravenous drug use was diagnosed in 1989.<sup>16</sup>

The advent of HIV infection in the black heterosexual population was shown by tests of antenatal blood samples. Between May 1987 and July 1988 infection rates were  $0.10 \pm 0.01\%$  among black people,  $0.09\%$  ( $0.03 - 0.21\%$ ) among coloured people,  $0\%$  ( $< 0.4\%$ ) among Indian people, and  $0\%$  ( $< 0.03\%$ ) among white people.<sup>11</sup> (All errors are 95% confidence limits.) In Johannesburg a survey of infection rates in male and female STD clinic attendees and female family planning clinic attendees carried out in 1990, gave prevalences of 1.8%, 2.7% and 1.1% respectively.<sup>17</sup> While the prevalences of HIV infection were considerably lower than in equivalent populations in East Africa at that time, there was little room for complacency.

In the mid-1980s the mining industry employed approximately 750 000 workers, most of whom were migrants either from rural areas within South Africa or from neighbouring states. Because most of these men live in single-sex hostels without wives or families, they clearly represent a high-risk group. Two surveys were done in 1986. The first, involving 1 200 women providing companionship



to miners working on several different gold mines, found no cases of HIV infection.<sup>11</sup> The second, involving mine-workers, found that among Malawians the prevalence was 4%, among those from Botswana it was 0.3% while among those from Lesotho, Mozambique, Swaziland and South Africa it was less than 0.1%.<sup>18</sup> Unfortunately, this very important set of data appears never to have been fully analysed or presented. While it led to the recommendation that the mines develop a preventive educational programme, it also led to the recommendation that since the 'prevalence of the disease has been established [the surveillance programme would] be suspended for white and black employees alike'.<sup>18</sup> In the early stages of an epidemic of HIV infection the doubling time for the incidence of HIV infection is usually about 1 year so that 10 years is sufficient to increase the prevalence 1 000 times. In 1986 it should have been clear that there was a window of about 5 years during which time effective measures would have had to be implemented if the spread of the disease were to be contained.

The extent of migrant labour in South Africa meant that once HIV infection had taken hold in the urban areas it would inevitably spread to rural areas. In the late 1980s prevalences among rural people were still low.<sup>17,19,20</sup> By 1993, however, seroprevalence among women attending antenatal clinics in Hlabisa, KwaZulu, was  $7.9 \pm 2.0\%$  with an estimated doubling time of 16 months.<sup>21</sup>

In order to understand the current state of the epidemic and to make reliable predictions it is essential to determine the current prevalence, the incidence and the rate at which the disease progresses in infected people. Experience from other developing countries shows that once the disease is established in a given population, the prevalence of HIV and the incidence of AIDS increase exponentially. Later in the epidemic, the rate of increase slows as the proportion of susceptible people declines<sup>22</sup> and the epidemic changes the demographic structure of the population.

In 1990 the doubling time of HIV prevalence was  $8.5 \pm 1$  months among black heterosexuals, between 7 and 21 months among pregnant women at Baragwanath Hospital, Johannesburg,<sup>23</sup> and 11, 10 and 7 months among men and women attending STD clinics and women attending family-planning clinics, respectively.<sup>17</sup> In 1994, nationwide surveys of antenatal clinics<sup>24</sup> gave prevalences ranging from a low of about 0.7% in Venda and the Cape to 4.8% in KwaZulu-Natal, with doubling times in all regions of approximately 1 year.

In 1990 Schall published models<sup>25</sup> to predict the course of the HIV epidemic over the next 30 years among men and women aged 15 - 49 years. The prevalence of HIV infection (Schall's scenario 2) increases exponentially in the early 1990s, increases more slowly through the late 1990s and reaches peak values in the region of 40 - 50% in the early years of the next decade. Schall argues that this is a worst-case scenario but believes that prevalences could reach 30% in the early years of the next decade. In 1993 the prevalence of HIV infection among women of child-bearing age (15 - 49 years) was estimated to be 4.7%<sup>26</sup> and the incidence of reported AIDS cases to be 67 per million per year, with doubling times of 14 months and 13 months respectively. These data are consistent with Schall's model for the early years of the epidemic.

## Social science

The social science papers cover a wide and fragmentary range of topics, samples and methodologies so that only some broad trends are outlined here. Lindegger and Wood<sup>27</sup> categorise the development of HIV/AIDS in four stages: (i) the predisposition to and risk of HIV; (ii) becoming infected with HIV; (iii) the development of end-stage symptoms; and (iv) AIDS-related death. Almost all the social science papers deal with stage 1, with some notable exceptions. Schlebusch and Cassidy<sup>28</sup> consider the influence of psychosocial co-factors on the course of the disease and, in particular, the importance of psychosocial stress, social support and emotional adjustment in the progression of the disease while Isaacs and Pegge<sup>29</sup> focus on psychotherapy and discuss the value of crisis intervention as a therapeutic approach in dealing with people with HIV/AIDS.

Most papers are concerned with the first of the four stages outlined above, insofar as they deal with issues that relate directly or indirectly to HIV/AIDS-related knowledge, attitudes and behaviours of various groups. These groups can be broadly divided into: (i) people involved in administering HIV/AIDS interventions (including health professionals in general,<sup>30,31</sup> social workers<sup>32,33</sup> and teachers<sup>34</sup>); and (ii) groups of people considered to be at risk of HIV infection (such as school pupils and adolescents,<sup>35-37</sup> university students,<sup>38</sup> street children,<sup>39</sup> and STD clinic attendees<sup>40</sup>). While some studies in the second category only measured knowledge and attitudes, others also studied reported behaviour. The diverse range of samples and measuring instruments makes it difficult to generalise, but several studies have found that, despite high levels of knowledge about HIV/AIDS, mineworkers,<sup>41</sup> street-children<sup>39</sup> and university students<sup>38</sup> still indulged in a range of high-risk sexual behaviours. These findings are consistent with a substantial social psychological literature,<sup>42</sup> which suggests that changing people's knowledge and attitudes is insufficient to change their behaviour.

The weak relationship between perceived health risk and preventive health behaviour presents particular challenges for social science researchers concerned with developing predictive models of high-risk behaviours that go beyond the influential but flawed knowledge-attitudes-practices (KAP) framework, which views knowledge and attitudes as primary determinants of behaviour. The reviewed literature provides some interesting starting points which highlight other co-factors that determine unsafe sexual behaviour. For example, Perkel *et al.*<sup>43</sup> suggest that sexual self-concept is a key mediator between HIV/AIDS-related knowledge and behaviour and argue that people with a low sexual self-concept are more likely to indulge in unsafe sex. Several papers<sup>44,45</sup> consider socio-economic issues such as migrant labour and commercial sex work, which both create high-risk situations for contracting HIV/AIDS, but may be the only options for people from poor communities. Others consider the economic and cultural constraints on women that make them particularly powerless in the negotiation of sexual encounters.<sup>46</sup>

The weak correlation between people's knowledge and attitudes on the one hand, and behaviour on the other, has implications for the types of educational interventions that are likely to succeed in promoting HIV/AIDS preventive



technology described in this paper would have been helpful in these cases. In some regions in the Western Cape, such as the Breederivier, acquired resistance can be as high as 19.3% and in such areas application of this technology may be particularly valuable. Patients with a history of previous tuberculosis treatment also have an almost three times higher risk of presenting with drug resistance, and may be good candidates for screening.

Other groups of patients to be investigated could be HIV-infected patients, where the chances are high for the presence of drug-resistant isolates,<sup>17</sup> as well as contacts of patients with drug-resistant strains.

It is, however, important to note that there is always a possibility for non-detection of mutations using a molecular screening technique. Susceptibility testing can still be done where indicated. Since molecular diagnosis of drug resistance can decrease the time for screening for resistance, from 6 - 12 weeks to a few days, the limited application of this technology, as indicated, may contribute towards combating the spread of drug-resistant strains of *M. tuberculosis*.

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### REVIEW ARTICLE

## Academic research and HIV/AIDS in South Africa

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The HIV/AIDS epidemic in South Africa is developing approximately 10 years behind the epidemics in East and Central Africa. In 1990 the prevalence in most groups tested was still low, typically less than 1%, but it was already known that the doubling time was about 1 year and it was clear that within the next 5 years prevalences would reach 10 - 30% in some groups.<sup>1</sup> The years between 1990 and 1995 were a window of opportunity during which time the control and management of HIV could have greatly reduced the future spread of the disease. This opportunity has been lost and prevalences in many groups have reached the expected levels.

The most important factors leading to severe HIV epidemics are well known and include high rates of sexually transmitted diseases (STDs), opportunistic infections, especially tuberculosis, and a range of social factors such as migrancy, social and political instability, and disruption of social support mechanisms and family structures. It is likely that the prevalence of HIV and the incidence of AIDS will continue to increase over the next 5 years. At this critical juncture in South Africa's history we face the possibility of a major, preventable epidemic that will decimate the economically active population. Yet effective interventions, through the control of STDs and effective, participatory, community-based education programmes that promote low-risk sexual behaviour, could have a dramatic effect on the future course of the epidemic.

The evidence, so far, is that none of the scientific community's attempted interventions has significantly altered the course of the epidemic. In this paper we review the scientific literature on HIV/AIDS in South Africa to suggest areas in which more research needs to be done and ways in which scientists can contribute to the management of the epidemic.

In April 1995 we searched three bibliographical databases, MEDLINE, SOCLIT and PSYCHLIT, for articles containing the key-words 'AIDS' and/or 'HIV' and 'South Africa', and followed up references cited in the papers that were found. (Because of space constraints it has not been possible to cite all of the papers that were reviewed.) Fig. 1 shows the number of publications concerned with technical issues, epidemiology, social science, and policy and planning. Each category has received a similar amount of attention but social science studies appear mainly as full papers while policy issues have been debated largely in the 'letters' columns.

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